expression of a ligase from T. sp. (i.e. *Thermus* species) AK16D and the biochemical characterization of this high fidelity enzyme.

SUMMARY OF THE INVENTION

The present invention is directed to a thermostable ligase having 100 fold higher fidelity than T4 ligase and 6 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent the ligation junction.

Another aspect of the present invention is directed to a thermostable ligase having 50 fold higher fidelity than T4 ligase and 5 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base penultimate to the ligation junction.

Yet another aspect of the present invention is directed to a thermostable ligase having, in the presence of a Mn^{2+} cofactor, a 12 fold higher fidelity than wild-type *Thermus thermophilus* ligase, when sealing a ligation junction between a pair of oligonucleotide probes hybridized to a target sequence where there is a mismatch with the oligonucleotide probe having its 3' end abutting the ligation junction at the base immediately adjacent to the ligation junction.

The present invention also relates to a DNA molecule encoding the thermostable ligase as well as expression systems and host cells containing such DNA molecules.

Another aspect of the present invention relates to the use of the thermostable ligase in carrying out a ligase detection reaction process or a ligase chain reaction process.

The ligase detection reaction process, involves detecting a target nucleotide sequence which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations. This

involves providing a sample potentially containing a target nucleotide sequence which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations.

The method further includes providing one or more oligonucleotide probe sets, each characterized by (a) a first oligonucleotide probe having a target specific portion and (b) a second oligonucleotide probe having a target-specific portion. The oligonucleotide probes in a particular set are suitable for hybridization to a target nucleotide sequence which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations. The probes are also suitable for ligation together when hybridized adjacent to one another on the target nucleotide sequence, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample.

The sample, the one or more oligonucleotide probe sets, and the thermostable ligase are blended to form a ligase detection reaction mixture. The ligase detection reaction mixture is subjected to one or more ligase detection reaction cycles comprising a denaturation treatment and a hybridization treatment. In the denaturation treatment, any hybridized oligonucleotides are separated from the target nucleotide sequence. During the hybridization treatment, the oligonucleotide probe sets hybridize at adjacent positions in a base specific manner to their respective target nucleotide sequences, if present in the sample, and ligate to one another. This forms a ligation product sequence containing the target specific portions connected together with the ligation product sequences for each set being distinguishable from other nucleic acids in the ligase detection reaction mixture. The oligonucleotide probe sets may hybridize to a nucleotide sequence in the sample other than their respective target nucleotide sequences but do not ligate together due to a presence of one or more mismatches and individually separate during the denaturation treatment. The presence of ligation product sequences produced as a result of the target nucleotide sequence being present in the sample is then detected.

In the ligase chain reaction process of the present invention, the presence of a target double stranded nucleic acid formed from first and second complementary target nucleotide sequences is detected in a sample. The target double

stranded nucleic acid differs from other nucleotide sequences by one or more single base changes, insertions, deletions, or translocations.

This method involves providing a sample potentially containing a target double stranded nucleic acid formed from first and second complementary nucleotide sequence. This nucleic acid differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations.

The method further includes providing a first oligonucleotide probe set, characterized by (a) a first oligonucleotide probe having a target specific portion and (b) a second oligonucleotide probe having a target-specific portion. The oligonucleotide probes in the first set are complementary to the first target nucleotide sequence which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations. The probes are also suitable for ligation together when hybridized adjacent to one another on the first target nucleotide sequence, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample. The method of the present invention also requires providing a second oligonucleotide probe set, characterized by (a) a third oligonucleotide probe having a target specific portion and (b) a fourth oligonucleotide probe having a target-specific portion. The oligonucleotide probes in the second set are complementary to the second target nucleotide sequence which differs from other nucleotide sequences in the sample by one or more single base changes, insertions, deletions, or translocations. The probes of the second set are suitable for ligation together when hybridized adjacent to one another on the second target nucleotide sequence, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample.

The sample, the first and second oligonucleotide probe sets, and the thermostable ligase are blended together to form a ligase chain reaction mixture. The ligase chain reaction mixture is subjected to one or more ligase chain reaction cycles comprising a denaturation treatment and a hybridization treatment. During the denaturation treatment, any hybridized oligonucleotides are separated from the target nucleotide sequences. In the hybridization treatment, the oligonucleotide probe sets hybridize at adjacent positions in a base specific manner to their respective target